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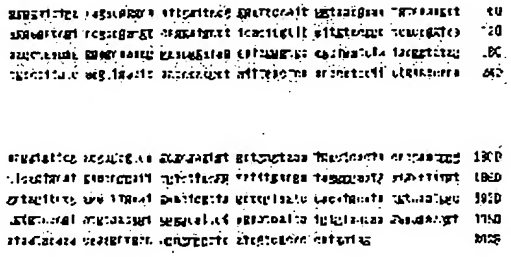
(54) PYRITHIAMIN-RESISTANT MARKER GENE AND TRANSFORMANT

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain the new subject marker gene comprising a pyrithiamin- resistant marker gene having a specific base sequence and expressing pyrithiamin resistance, and useful for creating a transformant using Aspergillus filamentous fungus and the like as a host cell.
SOLUTION: This new pyrithiamin-resistant marker gene comprises a DNA comprising a base sequence shown by the formula or a DNA comprising a base sequence in which one or several bases are substituted, deleted or added in the base sequence shown by the formula and expressing a similar pyrithiamin resistance as that of the DNA shown by the formula. The marker gene is useful as a selection marker for effectively selecting a transformant in breeding Aspergillus oryzae and the like in which Aspergillus filamentous fungus is major and utilized for production of a fermented food or various kinds of useful substances. The marker gene is obtained by culturing Aspergillus oryzae in a thiamine-free medium, selecting a pyrithiamin-resistant strain after treating the generated conidium with a mutagenic agent and constructing and screening of its genomic library.



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* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.

2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] The pyrithiamin resistance marker gene which consists of DNA of the following (a) or (b).
(a) DNA which consists of a base sequence expressed with the array number 1 of an array table.
(b) The pyrithiamin resistance marker gene which 1 or some bases consist of a permutation, deletion, or an added base sequence, and discovers the same pyrithiamin resistance as DNA (a) in a base sequence (a).
[Claim 2] The transformant which a transformation is carried out by the pyrithiamin resistance marker gene according to claim 1, and discovers pyrithiamin resistance.
[Claim 3] The transformant according to claim 2 whose host by whom introduces a pyrithiamin resistance marker gene and a transformation is done is Aspergillus (Aspergillus) group mold.
[Claim 4] The invention approach of the pyrithiamin resistance transformant characterized by including the following process.
(1) The process which reproduces ** KUTA containing a pyrithiamin resistance marker gene according to claim 1, the process which includes the duplicate vector obtained at the (2) above-mentioned processes in a host's chromosome, the process which chooses the host by whom the transformation was done to pyrithiamin resistance at the (3) above-mentioned processes under pyrithiamin existence.
[Claim 5] The invention approach of the transformant according to claim 4 which is ****** KUTA in which a duplicate vector contains genes of a different kind other than a pyrithiamin resistance marker gene according to claim 1.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the transformant which makes a host Aspergillus mold using the pyrithiamin resistance marker gene which consists of a new drug resistance gene, and this gene etc., and its invention approach.
[0002]
[Description of the Prior Art] The aspergillus which is mainly concerned with Aspergillus mold is extensively used for production of fermented foods, such as production of sake, white distilled liquor, and soy sauce, or various useful matter etc. In the breeding of the Aspergillus mold used for these industrial use, in order to give a desirable characteristic, the gene engineering-technique is very effective, and in order to choose a transformant efficiently, an usable selective marker is required.
[0003] As a drug resistance gene which can serve as a dominant marker of a transformant in mold Penicillium The phelomycin resistance gene of lock FORU tea (Penicillium roqueforti) (J.Biotccol 1996 Oct.18;51(1):97-105), benomyl of penicillium ice run JIYUMU (Penicillium islandicum) Resistance gene (curr.Genet.1995 Nov.;28(6)580-4), Aspergillus nigre (Aspergillusniger it is written as A. nigre below.) hygromycin B A resistance gene (Gene.1987;56(1):117-24) and an oligomycin resistance gene (Curr.Genet.1988 Jul;14(1):37-42), Aspergillus NIDORANSU (below Aspergillus nidulans A. it is written as NIDORANSU.) OLE OBASHIJIN A Resistance gene (JP,9-98784,A) Or Aspergillus The BENOMIRU resistance gene (Appl.Environ Microbiol 1990 Dec;56 (12):3686-92) of a hula bus (Aspergillus flavus) etc. is mentioned.
[0004] Current, aspergillus The gene which carries out the complementation of the auxotroph in the transgenics in A. ORIZE is used as a selective marker, and argB (2555 Agric. Biol.Chem. 51 (9), 2549-1987) or niaD (Gene 111 (2), 149-55, and 1992Feb 15) is mentioned as an example.
[0005]
[Problem(s) to be Solved by the Invention] However, in order to use the selective marker which carries out the complementation of the auxotroph, it is necessary to produce a nutrition requisite strain by mutation induction by UV irradiation, the source matter of variation, etc. And according to this random mutation induction, possibility of having a certain effect on the property of a host stock is high.
[0006] Moreover, the important aspergillus used in each brewing industry, such as sake, soy sauce, bean paste, or mirin, an Aspergillus Since ORIZE (it is written as below Aspergillus oryzae A. ORIZE.) shows strong resistance to extensive drugs, the effective drug resistance gene as a dominant marker is not yet discovered.
[0007] From the above reason A. The drug tolerance dominant selective marker which can modify a gene, without affecting the property was demanded to ORIZE.
[0008] Then, the technical problem of this invention is offering a pyrithiamin resistance gene applicable as a selective marker in gene engineering-use of Aspergillus mold. Moreover, this invention also makes it the technical problem to offer the transformant by which the characteristic was converted into the phenotype which is useful to genetic analysis with the vector containing such a drug resistance gene.
[0009]
[Means for Solving the Problem] Predetermined Aspergillus mold found out that sharp susceptibility was shown to the pyrithiamin which is the metabolic turnover **** analog of a thiamine, and the artificers of

this application changed into the resistant cell by performing variation processing to that susceptibility cell, they succeeded in isolating the gene (resistance gene) which gives pyrithiamin resistance from that resistant cell to A. ORIZE, and completed this invention.

[0010] That is, in invention of the 1st of this application, the above-mentioned technical problem was solved by adopting the means made into the pyrithiamin resistance marker gene which consists of DNA of the following (a) or (b).

(a) DNA which consists of a base sequence expressed with the array number 1 of an array table.

(b) The pyrithiamin resistance marker gene which 1 or some bases consist of a permutation, deletion, or an added base sequence, and discovers the same pyrithiamin resistance as DNA (a) in a base sequence (a).

[0011] Moreover, in invention of the 2nd of this application, the above-mentioned technical problem was solved by carrying out a transformation by the pyrithiamin resistance marker gene of the 1st invention of the above, and considering as the transformant which discovers pyrithiamin resistance. Moreover, in the above-mentioned invention, also when the host by whom a transformation is introduced and done makes a pyrithiamin resistance marker gene the transformant which is Aspergillus (Aspergillus) group mold, the above-mentioned technical problem can be solved.

[0012] Moreover, in invention of the 3rd of this application, it considered as the invention approach of the pyrithiamin resistance transformant characterized by including the following process.

(1) The process which reproduces ** KUTA containing a pyrithiamin resistance marker gene according to claim 1, the process which includes the duplicate vector obtained at the (2) above-mentioned processes in a host's chromosome, the process which chooses the host by whom the transformation was done to pyrithiamin resistance at the (3) above-mentioned processes under pyrithiamin existence.

[0013] In the invention approach of this pyrithiamin resistance transformant, a duplicate vector may be ****** KUTA containing genes of a different kind other than the pyrithiamin resistance marker gene indicated by the array number 1.

[0014] When the gene which gives the pyrithiamin resistance of this invention, i.e., a pyrithiamin resistance marker gene, is included in the chromosome of the cell of Aspergillus mold, it discovers pyrithiamin resistance. Moreover, the transformant which has pyrithiamin resistance out of these cells is easily detectable by placing the Aspergillus mold which had the pyrithiamin resistance marker gene incorporated into a specific culture medium.

[0015] Such a pyrithiamin resistance marker gene is a gene in which the phenotype which is useful to genetic analysis clarified, and is utility also about a transformant with pyrithiamin resistance at genetic analysis.

[0016]

[Embodiment of the Invention] To the pyrithiamin susceptibility cell which shows sharp susceptibility to the pyrithiamin which is the metabolic turnover **** analog of a thiamine as mentioned above, by performing variation processing, the pyrithiamin resistance marker gene of this invention changes this into a resistant cell, and isolates and obtains the gene (resistance gene) which gives pyrithiamin resistance from that resistant cell to A. ORIZE.

[0017] As Aspergillus mold in which sharp susceptibility is shown to a pyrithiamin as [show / in Table 1] -- A. KAWACHI ORIZE, A. nigre, and Aspergillus (Aspergillus lutiensis mut.kawachi it is written as A. KAWACHI below.) Aspergillus AWAMORI (Aspergillus awamori it is written as A. AWAMORI below.) Aspergillus Sawyer (Aspergillus sojae it is written as A. Sawyer below.) Aspergillus SHIROUSAMI (Aspergillus usami mut.Shiro-usami it is written as A. SHIROUSAMI below.) -- or -- A. nidulans is mentioned.

[0018]

[Table 1]

試験菌及び試験細胞	MIC (μg/ml)
A. オリーゼ	0.05
A. ニガー	1.0
A. カワチ	1.0
A. アワモリ	1.0
A. ソーヤ	1.0
A. シロウサミ	1.0
A. ニドランス	0.01

[0019] Specifically, it is susceptibility at a pyrithiamin. Variation processing of A. ORIZE is carried out by nitrosoguanidine (below nitrosoguanidine [/ It is written as NTG.]), a genomic library is created about the obtained resistant strain, and the DNA fragment expressed with the restriction enzyme map of drawing 1 containing the pyrithiamin resistance gene (it is hereafter written as ptrA.) which is dominant mutation is isolated from the library.
[0020] As DNA which carries out the code of the pyrithiamin resistance gene (ptrA) of this invention, there is DNA expressed with the array number 1 of an array table.
[0021] And if DNA which carries out the code of the ptrA of this invention is included in a suitable vector, and creates ****** KUTA and the transformation of the host is carried out, it can give a host pyrithiamin resistance as a selective marker, and can choose a transformant easily with the drug tolerance using a pyrithiamin. As a vector for Aspergillus mold of this ****** KUTA, pDG3, pkBY2, pSa123, and pTAex3 can be used.
[0022] Moreover, as for ****** KUTA incorporating DNA which carries out the code of the ptrA, it is possible to make it hold to stability to Escherichia coli etc. In that case, it is PUC as a thing usable as ** KUTA. There are system ** KUTA, an Escherichia coli-Aspergillus mold shuttle vector, etc.
[0023] Especially, ptrA(s) of this invention are the Aspergillus mold of a single nucleus and polykaryotic, and the marker gene which can also give pyrithiamin resistance to a practical use aspergillus, and are very useful to the breeding of A. ORIZE extensively used for industrial production of sake, white distilled liquor, soy sauce, and the useful matter etc.
[0024] Furthermore, ptrA of this invention can be applied also to aspergilli other than A. ORIZE, and is useful also in the breeding of other Aspergillus mold, and gene engineering-use. Moreover, ** KUTA which has DNA which ptrA of this invention can give pyrithiamin resistance to A. ORIZE, and carries out the code of the ptrA is an object offered for the first time as a drug tolerance grant vector to A. ORIZE.
[0025]
[Example] [Example 1] At the following processes, cloning of the pyrithiamin susceptibility related gene (ptrA) of A. ORIZE origin was performed.
[0026] 1-a) It is 0.1 ppm to the separation pyrithiamin of the pyrithiamin resistant mutant of A. ORIZE. A. ORIZE which shows susceptibility Inoculation of the HL-1034 share was carried out to CD plate (Czapek-Dox agar, 1% glucose, 2% agger) which does not contain a thiamine, and it cultivated for five days at 30 degrees C. It filters with a glass filter (3 G3 type) after suspending the conidium which grew in the Tween 80 (tween 80) solution 0.1%, and is 8000rpm about a filtrate. The at-long-intervals alignment was carried out for 5 minutes, and conidia were collected.
[0027] It is NTG so that this conidium may be set to 2.4x107spores / ml. It suspends in a solution (4000 ppm NTG), and they are for 10 minutes and 50rpm at 30 degrees C. Mutation was guided by carrying out a both-way shaking. The survival rate at this time was about 2.2%.
[0028] A conidium is washed 3 times after mutation induction processing by the penetrant remover (0.1% tween 80, 0.01M phosphate buffer (pH7.0)), and it is 1.0x107 spores/plate. It inoculated on the plate for screening (Czapek-Dox agar, 1% glucose, and 1 ppm pyrithiamin) so that it might become, and it cultivated for 30 degrees C and four days. This obtained six shares of pyrithiamin resistant strains.
[0029] This resistant strain is 1000 ppm. Although resistance was shown also to the pyrithiamin, since it had susceptibility like the old stock to KISHIMIDO, amphotericin B, and clotrimazole to oligomycin and cyclo, it was presumed that it was not multiple drug resistance but resistance specific to a pyrithiamin.
[0030] 1-b) In the creation pyrithiamin resistant strain of the genomic library of a pyrithiamin resistant strain, especially, from stock PTR-26 with sufficient growth, genomic DNA was extracted by the following approaches and refined. That is, the harvest of the hypha cultivated one evening at 30 degrees C was carried out with the glass filter (3G1 type) by the YPD liquid medium (0.5%yeast ext., 1.0% bact pepton, 2% dextrin), and distilled water washed. Dehydrated the fungus body, and it was made to freeze by liquid nitrogen, and ground using the mortar. The equivalent bacteriolysis solution (2% SDS, 0.1M NaCl, 10 mM EDTA, 50 mM Tris-HCl (pH7.5)) was added after suspending the ground fungus body in TE solution (10 mM Tris-HCl (pH8.0), 1mM EDTA). The at-long-intervals alignment was carried out by 3000 rpm after 1-hour standing at the room temperature for 10 minutes, and supernatant liquid was collected. a phenol / chloroform / isoamyl alcohol (25/24/1) -- 3000 rpm equivalent **** and after stirring a tube up and down slowly -- at long intervals for 5 minutes -- the upper layers were collected with care. - After leaving 20-

degree-C ethanol for 10 minutes at an amount, in addition -80 degrees C 2.5 times, the at-long-intervals alignment was carried out for 15 minutes, and 3500 rpm and DNA which precipitated were dried. RNase The solution (1micro/ml RNaseGS (TAKARA SHUZO CO., LTD. make), TE solution) was added, and 37 degrees C kept it warm for 1 hour. Equivalent phenol / chloroform / isoamyl alcohol are added, and it stirs slowly, and is 15000rpm. The at-long-intervals alignment was carried out for 5 minutes, and the upper layers were collected. -80-degree-C ethanol of an amount was added 2.5 times with 3M sodium acetate (pH5.2) of 1/10 amount, and 15000rpm and centrifugal [for 15 minutes] recovered DNA after neglect for 10 minutes at -80 degrees C.

[0031] Refined genomic DNA 10microg Restriction enzyme Sau3A I By 0.156U, deproteinization was carried out by the phenol / chloroform / isoamyl alcohol after 37 degrees C and 1-hour partial decomposition processing, and ethanol precipitate was carried out. The partial decomposition DNA was applied to agarose electrophoresis 0.8%, and DNA of 4 - 10kb field was extracted and refined. After making pDHG25 ** KUTA [gene, the 98th volume, and 61st page - 67 pages (1991)] which carried out full decomposition by obtained DNA and BamHI connect with a DNA ligation kit (TAKARA SHUZO CO., LTD. make), the transformation of Escherichia coli DH5 was carried out, and the genomic library of a resistant strain was created.

[0032] The plasmid was collected and refined from Escherichia coli after culture 37 degrees C and overnight in 50ml (1% bacto trypton, 0.5% bacto yeast extractives, 0.5% sodium chloride, PH7.2) of LB culture media which contain 100 ppm ampicillin for the Escherichia coli which made this genomic library contain.

[0033] 1-c) The transformation of the plasmid of the genomic library origin of the pyrithiamin resistant strain which is a manifestation and the cloning above of a pyrithiamin resistance gene (ptrA), and was made and adjusted was carried out to 89 shares of A. nidulans FGSC A by the following approaches.

[0034] That is, it collected by CD culture medium which does not contain a thiamine for A. nidulans by filtering a hypha with a glass filter (3GI type) after 30 degrees C and the shaking culture during two days, and sterilized water washed. The fungus body was fully suspended after dehydration in the 10ml protoplast-ized solution [20mg [/ml] YATARAZE (Ozeki brewing company make), 0.8M NaCl, 10mM sodium phosphate buffer solution, and pH6.0]. It was made to react for about 3 hours, shaking slowly at 30 degrees C. Glass filter 2000 rpm and centrifugal [for 5 minutes] recovered the protoplast in the filtrate filtered by 3 G3, and it washed twice by 0.8M NaCl. After suspending a protoplast in Sol 1 (0.8M NaCl, 10mM CaCl [2], 10mM Tris-HCl, pH8.0) so that it may be set to 2x108 / ml, it was easy to add So1 2 (40%(w/v) PEG4000, 50mM CaC12, 50 mM Tris-HCl, pH8.0) of 0.2 capacity, and mixed. 0.2ml protoplast suspension is poured distributively and it is 10microg. The plasmid of the genomic library origin was added and it mixed well. For 30 minutes, after neglect, it was easy to add 1ml So12, and mixed with the ice temperature. It is left for 15 minutes at a room temperature, 8.5ml Soll is added, and they are after mixing and 2000rpm well. Centrifugal [for 5 minutes] recovered the protoplast. 0.2ml Soll was added, and after carrying in the center of the minimal medium (Czapek-Dox agar, 1% glucose, 0.8 M NaCl, and 20ppb a biotin, 2% agger) containing the pyrithiamin of 2 ppm, it carried out multistory [of the soft-agar-medium (Czapek-Dox agar, 1% glucose, 0.8M NaCl, 20 ppb biotin, and 2 ppm pyrithiamin, 0.5% agger) 5 ml which kept it warm at 45 degrees C]. It cultivated for 30 degrees C and five - seven days.

[0035] The colony increased on this plate was considered to have a plasmid containing a pyrithiamin resistance gene, and about six colonies produced it on the pyrithiamin content culture medium. Inoculation of this colony was carried out to the CD+bi culture medium (Czapek-Dox agar, 1% glucose, and 20ppb biotin) containing 2 ppm pyrithiamin, it was cultivated for 30 degrees C and two days, and recovery purification of all the DNA was carried out from the increased fungus body according to the DNA extract purification method stated to example 1-b. It is Escherichia coli DH5 at this DNA. The transformation was carried out and it applied to LB culture medium containing 100 ppm ampicillin. Plasmid DNA was adjusted from the produced Escherichia coli colony.

[0036] This plasmid is 2 kb. DNA is included and it was named p142-13. The restriction enzyme map of the DNA fragment containing this pyrithiamin resistance gene ptrA is as being shown in drawing 1 . Subcloning of this fragment is carried out to a pBIISK+ vector, and it is pptrAEH. It named. The base sequence is as being shown in the array number 1.

[0037] pyrithiamin sensitive strain HL-1034 which is the old stock of PTR-26 ptrA and based on the array here from -- as a result of comparing the base sequence of the wild type gene of ptrA which carried out cloning, in ptrA, it was checked that the 670th adenine in the array number 1 has permuted by the guanine.

This a little salt radical permutation was presumed to be the cause of resistance-izing over a pyrithiamin by this.
[0038] [Example 2] Transformation 2-a which used Aspergillus mold for the host Plasmid p142-13 or pDHG25 vector acquired by transformation example 1-c of the Aspergillus mold using an isolation mold plasmid was introduced to A. ORIZE or A. nidulans by the transformation approach shown by example 1-c. After carrying in the center of the minimal medium (Czapek-Dox agar, 1% glucose, 0.8M NaCl, 2% agger) containing each concentration pyrithiamin, it carried out multistory [of the soft-agar-medium (Czapek-Dox agar, 1% glucose, 0.8 M NaCl, 0.5% agger) 5 ml containing the pyrithiamin of each concentration which kept it warm at 45 degrees C]. The pyrithiamin resistant strain was obtained after culture for 30 degrees C and five - seven days.
[0039] As shown in Table 2, A. ORIZE and A. nidulans can grow the transformant by p142-13 in all pyrithiamin concentration. On the other hand, the transformant by pDHG25 vector is 0.1 ppm. Susceptibility was shown also to the pyrithiamin. Therefore, it was checked that ptrA can be used as an effective selective marker over an aspergillus.
[0040]
[Table 2]

		PT 添加濃度 (ppm)			
	試験菌及び試験細胞	0	0.1	1.0	10
形質転換区分	A. オリゼー	++	++	++	++
	A. ニドランス	++	++	++	++
非形質転換区分	A. オリゼー	++	-	-	-
	A. ニドランス	++	-	-	-

[0041] 2-b) Plasmid pptrAEH acquired by transformation example 1-c of the Aspergillus mold using a chromosome embedded-type plasmid Or the transformation was carried out by the approach which stated A. ORIZE, A. nidulans, or A. nigre by example 1-c using pBIISK+. By the pyrithiamin addition minimal medium of each concentration which stated the protoplast which carried out transformation processing by example 2-a, it cultivated for 30 degrees C and five - seven days, and the pyrithiamin resistant strain was obtained.
[0042]
[Table 3]

		PT 添加濃度 (ppm)			
	試験菌及び試験細胞	0	0.1	1.0	10
形質転換区分	A. オリゼー	++	++	++	++
	A. ニドランス	++	++	++	++
	A. ニガー	++	++	++	++
非形質転換区分	A. オリゼー	++	-	-	-
	A. ニドランス	++	-	-	-
	A. ニガー	++	-	-	-

[0043] It is pptrAEH as shown in Table 3. The transformant of A. ORIZE and A. nidulans to depend, or A. nigre was able to be grown in all pyrithiamin concentration. On the other hand, the transformant by pBIISK+ is 0.1 ppm. Susceptibility was shown also to the pyrithiamin. Therefore, it was checked that ptrA can be used as an effective selective marker over Aspergillus mold.
[0044]
[Effect of the Invention] Since this invention considered as the pyrithiamin resistance marker gene which consists of DNA of the base sequence expressed with the array number 1, it gives resistance to the living thing which shows susceptibility to a pyrithiamin, and has the advantage that a pyrithiamin resistance gene applicable as a selective marker in the case of gene engineering-use of a gene engineering-breeding, genetic information analysis, etc. can be offered.
[0045] Moreover, the pyrithiamin resistance marker gene of this invention does not have effect like [when mutation induction is carried out to host stocks, such as a practical use aspergillus,], and can give a characteristic called the drug tolerance as a marker.
[0046] The transformant introduced in ** KUTA for chromosome recombination which used useful

pyrithiamin resistance as the selective marker in transgenics of an Aspergillus, especially an industrial use aspergillus by this invention, its invention approach, and the transformant obtained by the approach are offered. These can be used effective in the invention of the transformant for useful protein manufacture, a breeding, etc.

[0047] In transgenics, it especially A. ORIZE is not only widely used as an industrial use aspergillus, but is an aspergillus with high safety. Therefore, the pyrithiamin resistance gene of A. ORIZE origin is very useful because of the transformant creation for the breeding of A. ORIZE, and useful protein manufacture.

[0048]

[Layout Table]

SEQUENCE LISTING<110> Hakutsuru-Shuzou-Kabushiki kaisha <120> Pyrithiamine resistance-marker-gene and-transformed-cell<130> KP05407-14<160> 1 <210> 1 <211> 2028<212> DNA <213> Aspergillus oryzae<400> 1 ggggatctga cagacgggca attgattacg ggatcccatt ggtaacgaaa tgtaaaagct 60aggagatcgt ccgccgatgt caggatgatt tcacttgttt cttgtccggc tcaccggtca 120 aagctaaaga ggagcaaaag gaacggatag aatcgggtgc cgctgatcta tacggtatag 180 tgcccttatc acgttgactc aacccatgct atttaactca acccctcctt ctgaacccca 240 ccatcttctt ccttttcctc tcatcccaca caattctcta tctcagattt gaattccaaa 300 agtcctcgga cgaaactgaa caagtcttcc tcccttcgat aaacctttgg tgattggaat 360 aactgaccat cttctatagt tcccaaacca accgacaatg taaatacact cctcgattag 420 ccctctagag ggcatacgat ggaagtcatg gaatactttt ggctggactc tcacaatgat 480 caaggtatct taggtaacgt ctttggcgtg ggccggtgtt cgttcccagt catcgatgca 540 ttcacatgcc ctccctaagc tgggccctag actctaggat cctagtctag aaggacatgg 600 catcgatgga ctgggttcgt tctgagatta tacggctaaa acttgatctg gataatacca 660 gcgaaaaggg tcatgccttc tctcgttctt cctgttgatg gaatggctaa cagatgatag 720 tcattgcaac ttgaaacatg tctcctccag ctgccatcta cgaacccact gtggccgcta 780 ccggcctcaa gggtaaggtc gtggtttctg agaccgtccc cgttgaggga gcttctcaga 840 ccaagctgtt ggaccatttc ggtggcaagt gggacgagtt caagttcgcc cctatccgcg 900 aaagccaggt ctctcgtgcc atgaccagac gttactttga ggacctggac aagtacgctg 960 aaagtgacgt tgtcattgtt ggtgctggtt cctgcggtct gagcactgcg tacgtcttgg 1020ccaaggctcg tccggacctg aagattgcta tcgtcgaggc cagcgtctct cctggtcagt 1080agtccatgat ggattgcctt gcactcagct ttccggaact aacgtgcaat aggtggcggt 1140gcctggttgg gtggccaact cttttctgct atggtcatgc gccgtcccgc ggaagtcttc 1200ctgaacgagc tgggtgttccttacgaagag gacgcaaacc ccaactacgt tgtcgtcaag 1260cacgcctccc tgtttacctcgacactcatg tcgaaggttc tctccttccc caatgtcaag 1320ctcttcaatg ctaccgctgt tgaggacttg atcacccgtc cgaccgagaa cggcaacccc 1380cagattgctg gtgttgtcgt caactggacg ctggtcaccc ttcaccacga tgatcactcc 1440tgcatggacc ccaacactatcaacgctcct gtcatcatca gtaccactgg tcacgatggg 1500ccattcggcg ccttctgtgcgaagcgcttg gtgtccatgg gcagcgtcga caagctaggt 1560ggcatgcgtg gtctcgacat gaactcggcc gaggatgcca tcgtcaagaa cacccgcgag 1620gttactaagg gcttgataat cggcggtatg gagctgtctg aaattgatgg ctttaaccgc 1680 atgggcccta ccttcggtgccatggttctc agtggtgtca aggctgccga ggaggcattg 1740 aaggtgttcg-acgagcgtca gcgcgagtgt-gctgagtaaa tgactcacta-cccgaatggg 1800ttcagtgcat gaaccggatt tgtcttacgg-tctttgacga taggggaatg-atgattatgt 1860gatagttctg agatttgaat gaactcgtta-gctcgtaatc cacatgcata-tgtaaatggc 1920tgtgtcccgt atgtaacggt ggggcattct-agaataatta tgtgtaacaa gaaagacagt 1980 ataatacaaa caaagatgca agagcggctc atcgtcaccc catgatag 2028

[Translation done.]

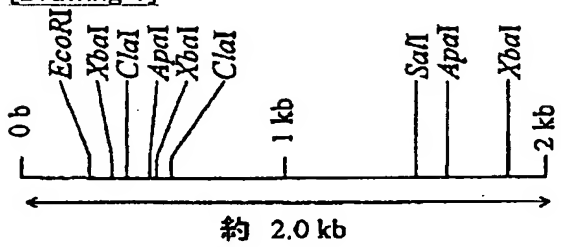
* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.

2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DRAWINGS

[Drawing 1]



[Translation done.]